

## CHOICE OF MEASUREMENTS FOR THE STATE OBSERVABILITY OF FERMENTATION PROCESSES

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### ABSTRACT

We present a preliminary simulation study on the observability of fermentation processes using simple sensors – a pH meter, a dissolved oxygen (DO) meter, a thermometer and a CO<sub>2</sub> meter. Our results show that, at least for the penicillin fermentation process, those measurements alone cannot give the state observability.

**KEYWORDS:** Observability, Fermentation, Bioreactor, Penicillin, Kalman Filter

### INTRODUCTION

Fermentation processes can be modeled with a set of coupled differential equations that describe the progress of the activity or state in a bioreactor such as the growth of microbes, consumption of nutrients and the generation of products [4], [5]. These state equations may be the ones either inspired from the kinetic models of biological interaction or based on black-box regression-type parametric models. In either case, various parameters present in the state equations are highly dependent on the strains or bioreactors used in a particular fermentation process, and therefore need to be identified experimentally. Once those parameters in the state equation are identified, we can use the model to control the input variables such as the feed rate of nutrients, temperature or pH to maximize the product generation [6], [7], [8].

However, such an optimal control of input variables requires the knowledge of the state variables  $\mathbf{x}$  in real-time. Some state variables such as pH may be directly measurable, while others such as intracellular enzyme concentration cannot be measured at all in real time. Still, another possibility is to measure some other variables  $\mathbf{z}$  such as optical density, instead of the state variables themselves. In this case however we need a knowledge of the nonlinear observation equation  $\mathbf{z} = \mathbf{h}(\mathbf{x})$ . Although optimal control techniques of the fermentation process have been studied for several decades, the lack of adequate real-time sensors and/or accurate observation equations prohibit biotechnology industry from adopting this mathematical technique.

In this regard, an important question is whether widely available sensors such as a pH meter, dissolved oxygen (DO) meter, thermometer and CO<sub>2</sub> meter may be usable to observe the state variables [1], [2], [10]. This observability issue is, of course process dependent, and there is no general comprehensive theory developed so far to check if a given nonlinear state equations and observation equation are observable or not. The purpose of this paper is to address this question through a simulation study in the case of penicillin fermentation process [3], [7], whose state equations have been developed by Birol, Uendey and Cinar [3]. Namely, we use an extended Kalman filter (EKF) [2], [9] to estimate the state vector, with the pH, DO, temperature (T), and CO<sub>2</sub> measurements, and check if there is a diverging element in the state error covariance matrix. If any element in the state error covariance matrix becomes infinity, then it indicates that the

corresponding subspace of the state vector space is unobservable with the given measurement set.

Although our approach is not general nor analytic, we hope that we could gradually gain some insight on the general observability condition through such particular simulation studies.

## STATE EQUATIONS

The majority of known fermentation process models do not take pH, DO, T and CO<sub>2</sub> as state variables, perhaps because their dependencies with standard state variables such as product or substrate concentrations are generally unknown or inaccurate. In this paper, we assume that the model in [3] is indeed accurate, and that an accurate model can always be found through a proper system identification methodology. For simplicity, we shall consider the batch, rather than the fed-batch, fermentation process of penicillin production. As has been done in [3], let us define the followings as the state variables:

$$x_1 = X, \text{ biomass concentration in [g/L]},$$

$$x_2 = H^+, \text{ hydrogen ion concentration in [mole/L]}$$

$$x_3 = P, \text{ penicillin concentration in [g/L]}$$

$$x_4 = S, \text{ substrate concentration in [g/L]}$$

$$x_5 = C_L, \text{ dissolved oxygen concentration in [mmole/L]}$$

$$x_6 = Q, \text{ generated heat in [cal]}$$

$$x_7 = T, \text{ temperature in Kelvin}$$

$$x_8 = CO_2, \text{ carbon dioxide concentration in [mmole/L]}$$

It will be convenient to organize the above state variables as a state vector

$$\mathbf{x} = [x_1, x_2, x_3, x_4, x_5, x_6, x_7, x_8]^T$$

Then it is known [3] that the following set of coupled first-degree differential equations can describe the progress of the fermentation process:

$$\frac{dx_1}{dt} = \mu x_1, \quad \mu = \left[ \frac{\mu_x}{1 + \frac{k_1}{x_2} + \frac{x_2}{k_2}} \right] \cdot \frac{x_4}{k_x x_1 + x_4} \cdot \frac{x_5}{k_{ox} x_1 + x_5} \cdot k_G \exp\left[-\frac{E_G}{R x_7}\right] - k_D \exp\left[-\frac{E_D}{R x_7}\right]$$

$$\frac{dx_2}{dt} = \gamma \cdot \mu x_1$$

$$\frac{dx_3}{dt} = \mu_{PP}x_1 - k \cdot x_3 \quad \mu_{PP} = \mu_p \cdot \frac{x_4}{k_p + x_4 + x_4^2/k_I} \cdot \frac{x_5^p}{k_{OP}x_1 + x_5^p}$$

$$\frac{dx_4}{dt} = -\frac{\mu}{y_{X|S}}x_1 - \frac{\mu_{PP}}{y_{P|S}}x_1 - m_X x_1$$

$$\frac{dx_5}{dt} = -\frac{\mu}{y_{X|O}}x_1 - \frac{\mu_{PP}}{y_{P|O}}x_1 - m_O x_1 + k_{LA}(x_{5,0} - x_5)$$

$$\frac{dx_6}{dt} = r_{Q1}\mu \cdot vx_1 + r_{Q2}vx_1$$

$$\frac{dx_7}{dt} = \frac{1}{v\rho_{CP}}x_6$$

$$\frac{dx_8}{dt} = \alpha_1\mu x_1 + \alpha_2 x_1 + \alpha_3$$

where the initial state vector is assumed to be as in [3]:

$$\mathbf{x}_0 = [0.1, 10^{-5.1}, 0, 15, 1.16, 0, 297, 0.5]^T$$

and the parameter values are as follows:

$$R = 1.9872041 \text{ [cal/K} \cdot \text{mole] }, \quad x_{5,0} = 1.16 \text{ [mmole/L] }, \quad \mu_x = 0.092 \text{ [h}^{-1}] , \quad k_1 = 10^{-10} \text{ [mole/L]}$$

$$k_2 = 7 \times 10^{-5} \text{ [mole/L] }, \quad k_X = 0.15, \quad k_{OX} = 2 \times 10^{-2}, \quad k_G = 7 \times 10^3, \quad k_D = 10^{33},$$

$$E_G = 5100 \text{ [cal/mole] }, \quad E_D = 50000 \text{ [cal/mole] }, \quad \gamma = 10^{-5}, \quad \mu_p = 0.005 \text{ [h}^{-1}] , \quad k = 0.04 \text{ [h}^{-1}]$$

$$k_p = 0.0002 \text{ [g/L] }, \quad k_I = 0.1 \text{ [g/L] }, \quad k_{OP} = 5 \times 10^{-4}, \quad p = 3, \quad Y_{X|S} = 0.45, \quad Y_{P|S} = 0.9,$$

$$m_X = 0.467 \text{ [h}^{-1}] , \quad Y_{X|O} = 0.04, \quad Y_{P|O} = 0.2, \quad m_O = 0.467 \text{ [h}^{-1}] , \quad \rho_{CP} = 1500 \text{ [cal/LC]},$$

$$\alpha_1 = 0.143 \text{ [mmole/g] }, \quad \alpha_2 = 4 \times 10^{-7} \text{ [mmole/h] }, \quad \alpha_3 = 10^{-4} \text{ [mmole/Lh] }, \quad k_{LA} = 40,$$

$$v = 100 \text{ [L]}$$

## STATE ESTIMATION

The first step of estimating the state variables using the EKF is to digitize the above differential state equations to get:

$$\mathbf{x}_{k+1} = \mathbf{f}(\mathbf{x}_k) + \mathbf{w}_k$$

If we use the simple Euler method, we shall have

$$x_{1,k+1} = x_{1,k} + \mu x_{1,k}$$

$$x_{2,k+1} = x_{2,k} + \gamma \mu x_{1,k}$$

$$x_{3,k+1} = x_{3,k} + \mu_{PP} x_{1,k} - k x_{3,k}$$

$$x_{4,k+1} = x_{4,k} - \frac{\mu}{y_{X|S}} x_{1,k} - \frac{\mu_{PP}}{y_{P|S}} x_{1,k} - m_X x_{1,k}$$

$$x_{5,k+1} = x_{5,k} - \frac{\mu}{y_{X|O}} x_{1,k} - \frac{\mu_{PP}}{y_{P|O}} x_{1,k} - m_O x_{1,k} + k_{LA} (x_{5,0} - x_{5,k})$$

$$x_{6,k+1} = x_{6,k} + r_{Q1} \mu v x_{1,k} + r_{Q2} v x_{1,k}$$

$$x_{7,k+1} = x_{7,k} + \frac{1}{v \rho_{CP}} x_{6,k}$$

$$x_{8,k+1} = x_{8,k} + \alpha_1 \mu x_{1,k} + \alpha_2 x_{1,k} + \alpha_3$$

$$F \square \frac{\partial \mathbf{f}}{\partial \mathbf{x}}$$

The next step is to get the Jacobian matrix,  $F \square \frac{\partial \mathbf{f}}{\partial \mathbf{x}}$  to propagate the state error covariance. The computation of  $F$  requires the partial derivatives of  $\mu$  and  $\mu_{PP}$ . Therefore, first let us consider  $\mu$ :

$$\mu = A_1 \cdot A_2 \cdot A_3 \cdot A_4 - A_5$$

where

$$A_1 = \frac{\mu_x}{1 + \frac{k_1}{x_2} + \frac{x_2}{k_2}}, \quad A_2 = \frac{x_4}{k_x x_1 + x_4}, \quad A_3 = \frac{x_5}{k_{ox} x_1 + x_5}, \quad A_4 = k_g \exp\left[-\frac{E_g}{R x_7}\right], \quad A_5 = k_d \exp\left[-\frac{E_d}{R x_7}\right].$$

$$\mu_i \square \frac{\partial \mu}{\partial x_i}, \quad i = 1, 2, \dots, 8$$

It is easy to check that are given as follows:

$$\mu_1 = A_1 \frac{-x_3}{(k_x x_1 + x_3)^2} k_x A_3 A_4 + A_1 A_2 \frac{-x_5}{(k_{ox} x_1 + x_5)^2} k_{ox} A_4, \quad \mu_2 = A_2 A_3 A_4 \frac{-\mu_x}{\left[1 + \frac{k_1}{x_2} + \frac{x_2}{k_2}\right]^2} \left[-\frac{k_1}{x_2^2} + \frac{1}{k_2}\right]$$

$$\mu_3 = 0, \quad \mu_4 = A_1 A_3 A_4 \frac{1}{(k_x x_1 + x_3)^2} \cdot k_x x_1, \quad \mu_5 = A_1 A_2 A_4 \frac{1}{(k_{ox} x_1 + x_5)^2} \cdot k_{ox} x_1$$

$$\mu_6 = 0, \quad \mu_7 = A_1 A_2 A_3 A_4 \cdot \frac{E_G}{R x_7^2} - A_5 \cdot \frac{E_D}{R x_7^2}, \quad \mu_8 = 0$$

$$\mu_{PP,i} = \frac{\partial \mu_{PP}}{\partial x_i}$$

Similarly, the partial derivatives of

$$\mu_{PP} = \mu_P \cdot A_6 \cdot A_7, \quad A_6 = \frac{x_4}{k_p + x_4 + x_4^2/k_I}, \quad A_7 = \frac{x_5^p}{k_{OP}x_1 + x_5^p}$$

are given by

$$\mu_{PP,1} = \mu_P \cdot A_6 \cdot \frac{-x_5^p}{(k_{OP}x_1 + x_5^p)^2} \cdot k_{OP}, \quad \mu_{PP,2} = 0, \quad \mu_{PP,3} = 0$$

$$\mu_{PP,4} = \mu_P A_7 \frac{(k_p + x_4 + x_4^2/k_I) - x_4(1 + 2x_4/k_I)}{(k_p + x_4 + x_4^2/k_I)^2}, \quad \mu_{PP,5} = \mu_P A_6 \frac{px_5^{p-1}(k_{OP}x_1 + x_5^p) - x_5^p p x_5^{p-1}}{(k_{OP}x_1 + x_5^p)^2}$$

$$\mu_{PP,6} = 0, \quad \mu_{PP,7} = 0, \quad \mu_{PP,8} = 0$$

$$F(\mathbf{x}) = \left( \frac{\partial f_i}{\partial x_j} \right) = (f_{i,j})$$

Then elements of the Jacobian matrix are given as

$$f_{1,1} = 1 + \mu_1 x_1 + \mu, \quad f_{1,2} = \mu_2 x_1, \quad f_{1,3} = 0, \quad f_{1,4} = \mu_4 x_1, \quad f_{1,5} = \mu_5 x_1, \quad f_{1,6} = 0, \quad f_{1,7} = \mu_7 x_1, \quad f_{1,8} = 0$$

$$f_{2,1} = \gamma(\mu_1 x_1 + \mu), \quad f_{2,2} = 1 + \gamma \mu_2 x_1, \quad f_{2,3} = \gamma \mu_3 x_1 = 0, \quad f_{2,4} = \gamma \mu_4 x_1, \quad f_{2,5} = \gamma \mu_5 x_1,$$

$$f_{2,6} = \gamma \mu_6 x_1 = 0, \quad f_{2,7} = \gamma \mu_7 x_1, \quad f_{2,8} = \gamma \mu_8 x_1 = 0$$

$$f_{3,1} = \mu_{pp1} x_1 + \mu_{pp}, \quad f_{3,2} = \mu_{pp2} x_1 = 0, \quad f_{3,3} = 1 + \mu_{pp3} x_1 - k = 1 - k, \quad f_{3,4} = \mu_{pp4} x_1, \quad f_{3,5} = \mu_{pp5} x_1,$$

$$f_{3,6} = \mu_{pp6} x_1 = 0, \quad f_{3,7} = \mu_{pp7} x_1 = 0, \quad f_{3,8} = \mu_{pp8} x_1 = 0$$

$$f_{4,1} = -\frac{\mu_{pp1}}{y_{X|S}} x_1 + \frac{\mu}{y_{X|S}} - \frac{\mu_{pp1}}{y_{P|S}} x_1 - \frac{\mu_{pp}}{y_{P|S}} - m_x, \quad f_{4,2} = -\frac{\mu_2}{y_{X|S}} x_1 - \frac{\mu_{pp2}}{y_{P|S}} x_1 = -\frac{\mu_2}{y_{X|S}},$$

$$f_{4,3} = -\frac{\mu_3}{y_{X|S}} x_1 - \frac{\mu_{pp3}}{y_{P|S}} x_1 = 0, \quad f_{4,4} = 1 - \frac{\mu_4}{y_{X|S}} x_1 - \frac{\mu_{pp4}}{y_{P|S}} x_1, \quad f_{4,5} = -\frac{\mu_5}{y_{X|S}} x_1 - \frac{\mu_{pp5}}{y_{P|S}} x_1,$$

$$f_{4,6} = -\frac{\mu_6}{y_{X|S}} x_1 - \frac{\mu_{pp6}}{y_{P|S}} x_1 = 0, \quad f_{4,7} = -\frac{\mu_7}{y_{X|S}} x_1 - \frac{\mu_{pp7}}{y_{P|S}} x_1 = -\frac{\mu_7}{y_{X|S}} x_1, \quad f_{4,8} = -\frac{\mu_8}{y_{X|S}} x_1 - \frac{\mu_{pp8}}{y_{P|S}} x_1 = 0$$

$$\begin{aligned}
f_{5,1} = & -\frac{\mu_1}{y_{X|O}}x_1 - \frac{\mu}{y_{X|O}} - \frac{\mu_{pp1}}{y_{P|O}}x_1 - \frac{\mu_{pp}}{y_{P|O}} - m_O, \quad f_{5,2} = -\frac{\mu_2}{y_{X|O}}x_1 - \frac{\mu_{pp2}}{y_{P|O}}x_1 = -\frac{\mu_2}{y_{P|O}}x_1, \\
f_{5,3} = & -\frac{\mu_3}{y_{X|O}}x_1 - \frac{\mu_{pp3}}{y_{P|O}}x_1 = 0, \quad f_{5,4} = -\frac{\mu_4}{y_{X|O}}x_1 - \frac{\mu_{pp4}}{y_{P|O}}x_1 = 0, \quad f_{5,5} = 1 - \frac{\mu_5}{y_{X|O}}x_1 - \frac{\mu_{pp5}}{y_{P|O}}x_1 + k_{LA}, \\
f_{5,6} = & -\frac{\mu_6}{y_{X|O}}x_1 - \frac{\mu_{pp6}}{y_{P|O}}x_1 = 0, \quad f_{5,7} = -\frac{\mu_7}{y_{X|O}}x_1 - \frac{\mu_{pp7}}{y_{P|O}}x_1 = -\frac{\mu_7}{y_{X|O}}x_1, \quad f_{5,8} = -\frac{\mu_8}{y_{X|O}}x_1 - \frac{\mu_{pp8}}{y_{P|O}}x_1 = 0, \\
f_{6,1} = & r_{q1}v\mu_1x_1 + r_{q1}v\mu + r_{q2}v, \quad f_{6,2} = r_{q1}v\mu_2x_1, \quad f_{6,3} = r_{q1}v\mu_3x_1 = 0, \quad f_{6,4} = r_{q1}v\mu_4x_1, \quad f_{6,5} = r_{q1}v\mu_5x_1, \\
f_{6,6} = & 1 + r_{q1}v\mu_6x_1 = 1, \quad f_{6,7} = r_{q1}v\mu_7x_1, \quad f_{6,8} = r_{q1}v\mu_8x_1 = 0, \\
f_{7,1} = & 0, \quad f_{7,2} = 0, \quad f_{7,3} = 0, \quad f_{7,4} = 0, \quad f_{7,5} = 0, \quad f_{7,6} = \frac{1}{v\rho_{CD}}, \quad f_{7,7} = 1, \quad f_{7,8} = 0, \\
f_{8,1} = & \alpha_1\mu_1x_1 + \alpha_1\mu + \alpha_2, \quad f_{8,2} = \alpha_1\mu_1x_1, \quad f_{8,3} = \alpha_1\mu_3x_1 = 0, \quad f_{8,4} = \alpha_1\mu_4x_1, \quad f_{8,5} = \alpha_1\mu_5x_1, \\
f_{8,6} = & \alpha_1\mu_6x_1 = 0, \quad f_{8,7} = \alpha_1\mu_7x_1, \quad f_{8,8} = 1 + \alpha_1\mu_8x_1 = 1
\end{aligned}$$

If we assume the state variables  $x_2, x_5, x_7, x_8$ , i.e.,  $H^+, C_L, T, CO_2$  are measured, then the observation matrix will be  $H_1$  below, On the other hand, if  $x_1, x_2, x_5, x_7, x_8$  are measured, then the observation matrix becomes  $H_2$ .

$$H_1 = \begin{bmatrix} 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \end{bmatrix}, \quad H_2 = \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \end{bmatrix}$$

Finally the EKF using the continuous state equation and the discrete observation equation can be described as follows:

**for** k=0,1,

$$R_{e,k} = R + HP_{k|k-1}H^T$$

$$\mathbf{e}_k = \mathbf{z}_k - H\hat{\mathbf{x}}_{k|k-1}$$

$$\hat{\mathbf{x}}_{k|k} = \hat{\mathbf{x}}_{k|k-1} + P_{k|k-1}H^TR_{e,k}^{-1}\mathbf{e}_k$$

$$P_{k|k} = P_{k|k-1} - P_{k|k-1} H^T R_{e,k}^{-1} H P_{k|k-1}$$

Solve  $\frac{d\mathbf{x}(t)}{dt} = \mathbf{f}(\mathbf{x}(t))$  for  $\mathbf{x}(t_{k+1}) \square \hat{\mathbf{x}}_{k+1|k}$  starting with  $\mathbf{x}(t_k) \square \hat{\mathbf{x}}_{k|k}$

$$P_{k+1|k} = F_{k+1} P_{k|k} F_{k+1}^T + Q$$

**end**

## SIMULATION RESULTS

Figure 1 compares the state estimates  $\hat{\mathbf{x}}_{k|k}$  obtained with  $H_1$  and  $H_2$  against the true  $\mathbf{x}(t)$  obtained by solving the differential equation. We can see that the results with  $H_1$  do not estimate the true state vector correctly, except the ones that are directly measured, whereas the addition of biomass measurement in  $H_2$  gives accurate state vector estimates.

## CONCLUSIONS

In this paper, we have studied the observability issue in the process modeling through a simulation study of the penicillin fermentation process. It has been found that the widely available sensors; pH meter, dissolved oxygen (DO) meter, thermometer and CO<sub>2</sub> meter are not adequate to make the process observable.

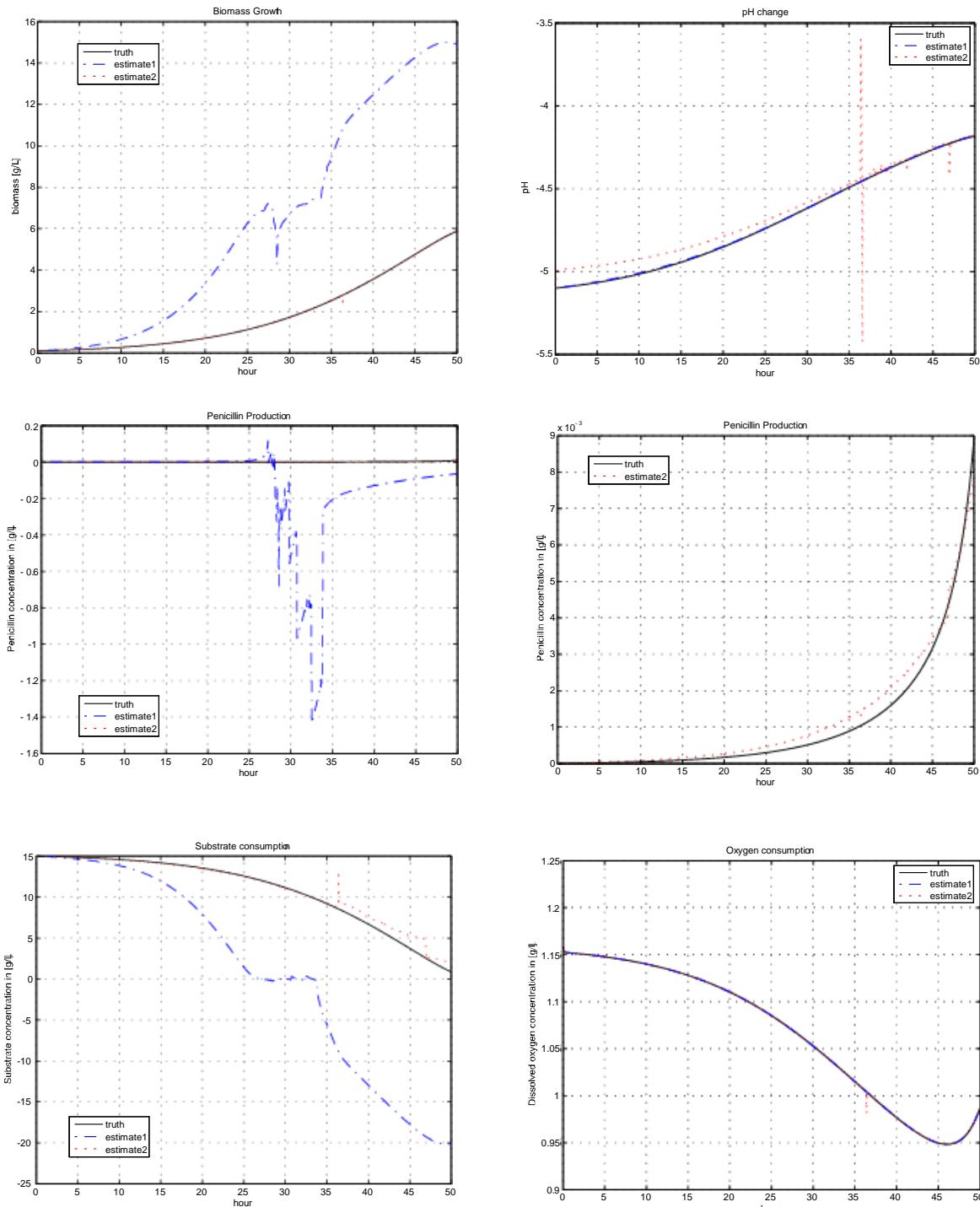
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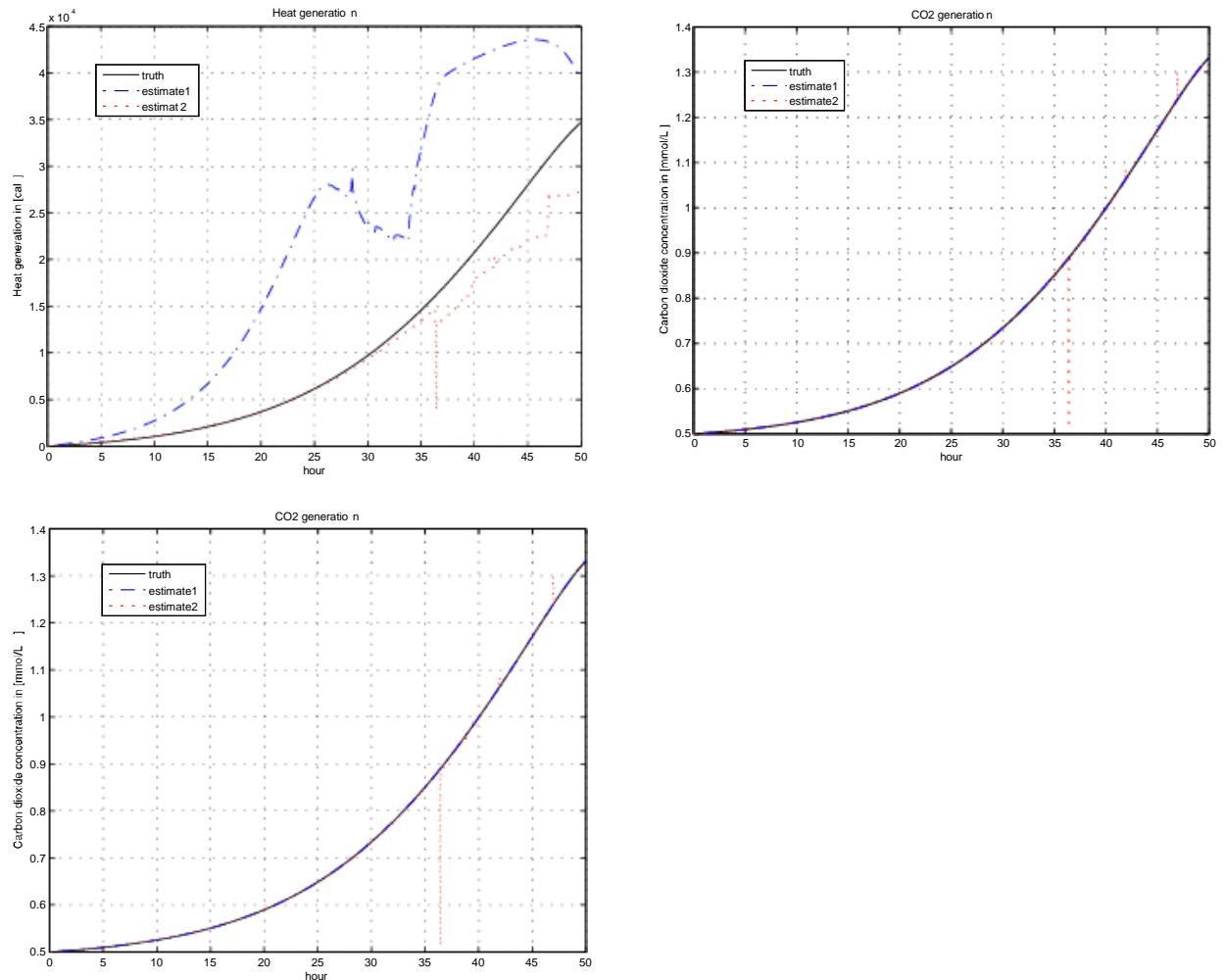
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## APPENDICES





**Figure 1: Comparison of State Estimates for Different Sets of Measurements**

